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STUDY OF A SPIROCHETE OBTAINED FROM A CASE OF RELAPSING FEVER IN MAN, WITH NOTES ON MORPHOLOGY, ANIMAL REACTIONS, AND ATTEMPTS AT CULTIVATION.*

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INTRODUCTION.

THE spirochetes studied were obtained from the blood of a patient in the service of Dr. Carlisle, in Bellevue Hospital.† By inoculation of monkeys and rats we have been able to keep the spirochetes alive through the continuous passage of blood from one animal to another. It is our purpose to record as briefly as possible the various observations which we have made upon the animal reactions, with a brief description of the morphology of the micro-organism, and the various attempts made to cultivate it outside the body. Our studies have continued over a period of five months.‡

SPIROCHETAL INFECTION IN MONKEYS.

Three monkeys of the species *Macacus Rhesus*, and two of the species *Macacus Javanus*, were used for inoculation experiments. These species were selected because of their known susceptibility to infection with *Sp. Obermeieri.* The disease was produced by subcutaneous inoculation of human or animal blood containing spirochetes.

Incubation period.—Following the injection there was noted an incubation period during which no spirochetes were found in the circulating blood. The duration of this was, in one monkey (Rhesus I), two days; in another (Rhesus III), three days; in a third (Java II), three days; in the remaining two, impossible to determine, because

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* Received for publication, February 7, 1906.

† Dr. Carlisle's clinical report of this case is found in this number of the *Journal.*

‡ Condensed reports of our work were given at the October and December meetings of the New York Pathological Society, and at the Society of American Bacteriologists at its December meeting of 1905, through the courtesy of Professor Novy.

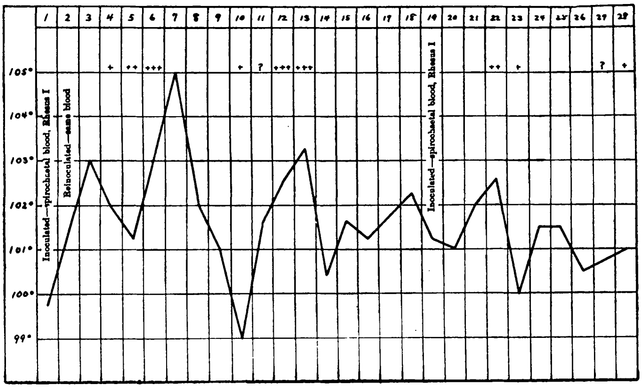
of double inoculations, but not exceeding three days in one (Java I), or six days in the other (Rhesus II). During this incubation period no obvious signs of illness were observed.

Relapses.—The disease, in monkeys, like relapsing fever in man, was characterized by a succession of paroxysms, each attended by the appearance of the spirochetes in the peripheral blood, and certain general symptoms of illness. Three of the monkeys showed three paroxysms; one monkey, four. The duration of the individual paroxysms, and of the intervals, varied widely. The longest period during which spirochetes were present in the blood on successive daily examinations was four days; the shortest, one day. The average duration of the paroxysms apparently slightly decreased with the progress of the disease. The longest interval noted was eight days; the shortest, two days. The average length of interval in general appeared to increase after the first. One marked exception to this was noted, the intervals being two, eight, and three days respectively. We see, therefore, that there is little regularity or uniformity in the onset, number, or duration of the paroxysms.

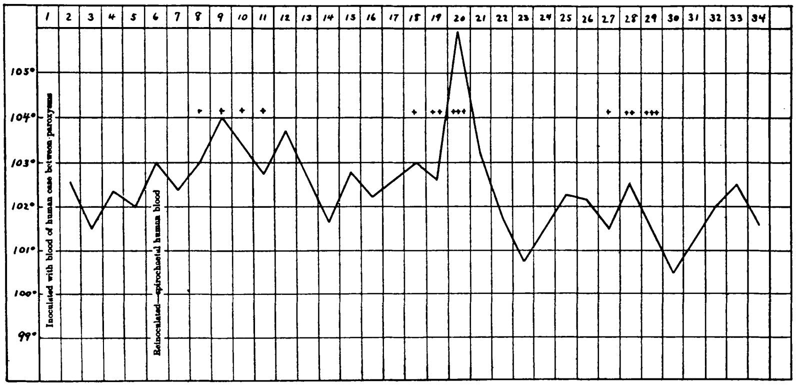
General symptoms.—A rise in temperature usually accompanies the appearance of the spirochetes in the blood, and persists until their disappearance. The temperature charts, however, are far less striking than those of relapsing-fever patients. There is normally considerable variation in the body temperature of different monkeys, and even from day to day in the same monkey. Thus Rhesus I, before the onset of his first paroxysm, showed a temperature ranging from 101° to 103° F.; Rhesus II, a temperature ranging from 99.8° to 102°; Rhesus III, a temperature between 99.6° and 104.2°; and Java I, between 99.8° and 102.8°. Moreover, the febrile reaction during the paroxysm, though usually present, is poorly marked and inconstant as contrasted with the typical fever curve in relapsing fever of man. It rarely exceeds 2° F. above the normal average. The highest recorded temperature is 106°, noted in Rhesus I toward the end of his second paroxysm.

The appended temperature charts show the general course of the fever. Their most impressive feature is the marked irregularity.

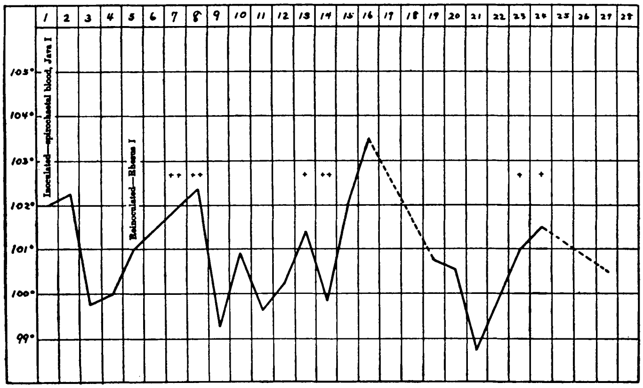
During the paroxysms the animals exhibit certain unmistakable signs of illness. When left alone, they sit huddled up against the



JAVA I.



RHESUS I.



RHESUS II.

wall of the cage, and from time to time cry shrilly, as if in pain, and, when disturbed, show an unwonted irritability. The appetite is poor during the attacks. In two monkeys there was a sharp diarrhea, lasting a few days. One monkey showed distinct, though transient, jaundice of the conjunctivæ. Edema of the eyelids was seen in Rhesus I during the latter part of his illness, and Java I developed a marked conjunctivitis of the right eye, with hemorrhage into the anterior chamber, during his second relapse.

Unfortunately, lack of time prevented us from carefully studying the blood changes during and after the paroxysms.* No differential or total leucocyte counts were made. Certain changes, however, attracted our attention in the routine examination of the blood smears for spirochetes. Very striking was the polychromatophilic degeneration of the red cells, which was constantly seen after the first appearance of the spirochetes in the blood. Granular degeneration, on the other hand, was not observed. The large mononuclears impressed us as being in excess, although without accurate differential counts, their exact numerical relation to the presence of the spirochetes in the circulating blood was not determined. Numerous very large forms, with pale, poorly staining nuclei, and ragged degenerated cytoplasm, were always present. The blood plaques appeared to be markedly increased.

The course of the disease in all monkeys was benign. The animals speedily regained their usual good health after the last paroxysm. During their illness they appeared to lose moderately in weight, especially Rhesus I and Java I.

SPIROCHETAL INFECTION IN WHITE RATS.

Up to the time of writing, about 100 rats have been inoculated. Two rats were inoculated on October 24, from Rhesus III; since this time the spirochetes have been kept alive by constant passage from rat to rat. From a few drops to 1.5 c.c. of blood, to which sodium citrate was added, was injected beneath the skin of the back. In all cases where the inoculated blood showed spirochetes at the time of injection, a spirillosis developed in the experimental animal. The only exceptions to this observation were noted in two diseased rats, which died on the second day after inoculation, and showed encapsulated abscesses of lung, chronic sepsis, and advanced secondary anemia.

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* The blood of each monkey examined before inoculation was found free from hematozoa or other blood parasites.

The incubation period ranged from one to five days, averaging about two and one-half days. It has diminished with the frequent passage from rat to rat. Thus the average time for the development of the disease in the last 15 rats inoculated was only about one day, as compared with three days in the first 15 animals used. The minimum incubation period was not determined with accuracy, because the majority of the rats were not examined until the third day. In Rat 89 numerous spirochetes were seen 19 hours after inoculation.

Owing to the fact that most of the rats were killed as soon as numerous spirochetes were found in the blood, the duration of the illness was determined in comparatively few. In those allowed to live, spirochetes were found in the peripheral circulation for a period varying from one to five days; most frequently, one to two days. It is an interesting fact that the single rat, killed after they had persisted for five days, showed multiple large chronic abscesses of the lung, from which we may infer, if we like, that his normal resistance was diminished.

No relapses were observed in two rats, whose blood was carefully studied for 12 and 18 days respectively. That relapses may not occur we cannot say definitely. The rats at no time betray any definite objective indications of illness, save the presence of the specific organisms in the blood. The same qualitative changes in the appearance of the blood cells—marked polychromatophilia, and large mononuclears, resembling bone-marrow cells, megakaryocytes—were noted as in the monkeys.

The only gross pathological change seen in animals killed during the height of infection was swelling of the spleen. No report of the histological examination of the organs will be attempted here. We must, however, state that repeated examinations of smears from the spleen, bone-marrow, and liver, stained by Wright's, Giemsa's, and other methods, failed to show spirochetes, either within or without the cells, at any stage of the disease, with the exception of a few extracellular single forms in one spleen smear. The large mononuclears, with vacuoles, we have seen in abundance. The vacuoles contained only granular detritus; nothing in the least suggestive of spirochetes. It is possible that, with the particular organism studied, phagocytosis, if it occur at all, plays an altogether unimportant rôle. Shortly after the disappearance of the spirochetes from the circulating blood, spleen smears were likewise examined without success. This is in marked contradiction to the findings of Metchnikoff,[1] Soudakewitch,[2] Sawtchenko and Melkich,[3] with the spirochetes of Obermeier. In splenic smears from artificially infected monkeys, spirochetes were distinctly seen and depicted within the polymorphonuclears (Metchnikoff) or the large mononuclear macrophages (Soudakewitch); in the latter they often lie coiled up within a vacuole in the cell protoplasm. Sawtchenko and Melkich observed a similar phagocytosis on the part of the endothelial cells, when spirochetal blood was injected into the peritoneal cavity of guinea-pigs.

Cantacuzène[4] in the spirillosis of geese Levaditi,[5] and Marchoux and Salimbeni,[6] in the spirillosis of fowls, likewise describe the phagocytosis which occurs in the spleen toward the end of the attack. In the drawings of the spleen smears by Cantacuzène a number of clearly defined, unmistakable spirochetes are shown lying coiled within the vacuoles.

Sawtchenko, in a monkey whose spleen had previously been removed, observed that the spirochetes became very numerous during the course of infection, and persisted, a chronic spirillosis being established, the animal finally dying. The spirochetes are shown in great numbers in the blood smears taken from the superior vena cava, and within the polymorphonuclears of the peripheral blood. Such pictures were not seen during the natural course of infection in our human cases, or in the monkey or rat.* Soudakewitch[2] and Iwanoff[7] noted the occurrence of phagocytosis in the circulating blood of patients with relapsing fever, near the crises, their statements varying in this respect from those made by other observers. Excision of the spleen is not followed in all cases by a chronic spirillosis, as shown in one of our rats (90), which was splenectomized 24 hours before inoculation. In this case the infection ran the usual course, the spirochetes appearing and disappearing one day later than in the control rat.

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* In the last edition of one of the noted American textbooks upon the clinical examination of the blood, we have been surprised to see the drawings and cuts of Sawtchenko reproduced, without any reference to their source, or any explanation that they were not drawn from human, but from the blood of the splenectomized monkey. In view of the fact that the spirochetes are never present in the blood of human being or monkeys in such abundance, and that the polymorphonuclear leucocytes in the circulating blood, according to most observers and ourselves, do not contain spirochetes, a most erroneous impression of the blood picture of relapsing fever is thereby conveyed to the reader.

SPIROCHETAL INFECTION IN RABBITS.

Animal Inoculated	Source of Infectious Material; Dose; How Inoculated	Remarks
Rabbit III, Sept. 19	1 c.c. of citrate blood from Java I, intravenously. (Smears positive)	Few spirochetes on following day; numerous spirochetes on second day; negative on succeeding days. No spirochetes in smears from liver, spleen, or bone-marrow
Rabbit IV, Sept. 19	0.5 c.c. of citrate blood fromJava I, intravenously	Few spirochetes on following day; negative on succeeding days
Rabbit V, Sept. 21	1 c.c. of citrate blood from Rabbit III, intravenously. (Smears sp. ++)	Negative
Rabbit VI, Oct. 2	0.5 c.c. of citrate blood from Rhesus I, intravenously. (Smears sp. ++)	Negative

As seen from the above table, two of the rabbits showed undoubted susceptibility to spirochetal infection, if we are to judge by the multiplication of the spirochetes in their blood. The animals did not appear ill, and the organisms persisted for only one or two days. There were no relapses. The one attempt to inoculate a second rabbit with the spirochetal blood of Rabbit III was unsuccessful. It may be remarked here that many articles on relapsing fever state specifically that the rabbit is not susceptible to infection with the *Sp. Obermeieri*, and we have met with no previous record of successful inoculations in rabbits, except that of Dutton and Todd.[8] This discrepancy may possibly be explained by the fact that young rabbits were used, and the material was injected intravenously. It is obvious, however, that rabbits are not well adapted for experimentation with the spirochetes, and in our further work white rats were used exclusively, because of their constant susceptibility and lesser cost.

SPIROCHETAL INFECTION IN GUINEA-PIGS.

Several guinea-pigs inoculated intraperitoneally failed to reveal spirochetes in the circulating blood.

SPIROCHETAL INFECTION IN WHITE MICE.

Two small white mice were inoculated with spirochetal blood from Rat 80. On the second day numerous spirochetes were found in the blood. No further observations on mice have been made.

IMMUNITY.

A. *Active immunity.*—Both in rats and monkeys previous infection confers immunity. We are unable to say how long this immunity persists, since our observations cover a period of only three or four months. We do know, however, that it has lasted for 94 days in the case of Rhesus I, 86 days in Rhesus II, and 64 days in Rhesus III. Inoculations with spirochetal blood (Rat 78) after these intervals were negative. The control monkey (Java II), inoculated with the same blood fom Rat 78, showed spirochetes in the blood on the third day. Only four observations have been made on rats, but those animals likewise could not be reinfected. Rats 4, 6, 41, and 46 were reinoculated after periods of 13, 10, 41 and 33 days respectively, following the last appearance of the spirochetes in their blood. Daily examinations for seven to eight days failed to show spirochetes.

B. *Passive immunity.*—In contrast to the perfect protection acquired through previous infection is the uncertainty of the protection furnished by the inoculation of serum from man and animals that have passed through a previous infection. The poor results obtained in our experiments upon the production of passive immunity are doubtless due to the fact that the serum used was not obtained from actively immunized animals—a statement in accord with the observations of Professor Novy,[9] who found that the injection of the blood from actively immunized rats not only prevented the infection in white rats, but caused the rapid disappearance of the spirochetes from the circulating blood.

We submit a synopsis of our experiments along this line, and the inferences which in our opinion may reasonably be drawn from them:

a) In these experiments serum and spirochetal blood were mixed before inoculation.

Experiment I.—Rat 22: Inoculated with 1 c.c. of blood from Rat 16, and 1 c.c. of normal human serum (kept five days at room temperature); in contact for five minutes before inoculation. Spirochetes appeared on the third day in considerable numbers.

Rat 24 (control): Inoculated with 1 c.c. of blood from Rat 16. Spirochetes appeared on the third day.

Rat 21 (control): Inoculated with 1 c.c. of blood from Rat 16. Spirochetes appeared on the third day.

Normal human serum exerted no inhibitory power upon the development of the infection.

Experiment II.—Rat 23: Inoculation with 1 c.c. of spirochetal blood from Rat 16+1 c.c. of human serum (A.M.P.) obtained 12 days after second and last relapse. (Serum kept for five days at room temperature.) Spirochetes appeared on the fifth day.

Rats 21, 24 (controls): See Experiment I.

The "immune" human serum apparently caused a retardation of the infection for two days.

Experiment III.—*Rat 25: Inoculated with 1 c.c. of blood from Rat 16 +1 c.c. of serum from Rhesus I, obtained 43 days after the third relapse, and 15 days after inoculation with spirochetal blood from Rat 1. (Serum kept 24 hours at room temperature.) Blood negative for nine days; not further examined.

Rats 21, 24 (controls): See Experiment I.

Complete protection was afforded in this case by the serum of a monkey after natural infection, and after a single subsequent inoculation with spirochetal blood.

b) In the following experiment the immune serum was injected previous to the inoculation with infective blood.

Experiment IV.—Rat 26: Inoculated subcutaneously with 20 gtt. of serum from Rhesus I, drawn 43 days after the last relapse, two days old. Three days later inoculated with 15 gtt. of spirochetal blood from Rat 21. Spirochetes appeared on the third day.

Rat 30 (control): Inoculated with spirochetal blood from Rat 21. Spirochetes appeared on second day.

c) In the following experiment an unsuccessful attempt was made to cause the disappearance of the spirochetes from the blood during a paroxysm, by the injection of the citrate blood of a rat which had passed through its infection. We were led to perform this experiment by the very striking and favorable results recorded by Professor Novy[8] in the use of blood from *actively* immunized rats.

Experiment V.—Java II: Inoculated with spirochetal blood from Rat 78. A few spirochetes appeared on the third day, and were very numerous and extremely motile on the fourth day. On the fourth day the monkey was inoculated subcutaneously with 5 c.c. of blood of Rat 75, withdrawn five days after last appearance of the spirochetes. Blood was drawn from the ear and examined every 15 minutes for one hour, and again after two, three, and five hours.

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* A similar set of experiments was performed five days previously. The results are not given in detail, because two of the rats escaped from their cages, and could not be positively identified. In this case the immune human and monkey serum afforded protection, whereas control rats and rat inoculated with normal human serum developed spirochetes on the third day.

No effect was produced, the spirochetes becoming more numerous and retaining their motility after five hours. On the following day, 24 hours later, no spirochetes were found. The first relapse occurred five days later.

It is evident from this experiment that the anti-bodies in the blood of a rat which has recovered from the spirochetal infection are not present in quantities sufficient to abort the infection in other animals. Furthermore, the inoculation of this serum did not inhibit or retard the onset of the first relapse, or shorten its duration.

It may be of interest to note an additional observation made at this time. Immune blood of the above rat (No. 78), as well as the immune blood of Rhesus III, when mixed with the spirochetal blood of Java II, and examined in hanging-drop, showed no agglutinating properties, nor was any decrease in the motility of the organisms observed, after several hours. (Apparent lack of "immobilisines" of Levaditi.) The only phenomenon noted was a marked tendency of the spirochetes to adhere to the red blood corpuscles. In the hanging-drop preparation, after 24 hours at room temperature, no spirochetes were found, however, whereas in the control hanging-drop, and with mixtures of Java II and Rat 75, numerous clumps of spirochetes were seen, only a few of the organisms being still motile. The dissolution or cytolysis of the spirochetes evidently took place slowly in serum.

MORPHOLOGY.

Because of the emphasis that has been laid upon the morphology of the spirochetes as a basis for their classification, by Schaudinn[10] and other writers, it seems worth while to enter into a somewhat minute description of the forms which the organisms studied by us may assume.

Under conditions of which we know practically nothing, these spirochetes may show the most astonishing variations—variations in length, in thickness, in the number and closeness of the spirals, in motility, and in arrangement.

The spirochete, as seen in smears, is a tapering, spiral filament, whose length varies roughly from one and one-half to 10 times the diameter of a red cell; exceptionally, even longer forms are seen. The breadth or thickness is more constant; but still one meets with very slender (attenuated) forms, on the one hand, and thick, almost ribbon-like or fusiform types, on the other. In some rats we have observed that the breadth varies markedly in different portions of one and the same spiral. The number and conformation of the spirals are subject to very great variations. The conditions which determine these variations in morphology we have not been able to ascertain positively. These marked variations are observed only when the spirals are most numerous; that is, within 24 hours previous to their disappearance from the circulating blood. The extremes are typified by a short organism, showing but a single sinuous curve; and a long one with 15 or more closely approximated spirals. Between these two there may be found every transitional form.

The tapering ends of the spirochetes in successful smears are usually drawn out to a considerable length, so that they may readily be interpreted as terminal whips or cilia. In special stains for cilia we have never seen more than one terminal filament.

In every stained preparation examined the majority of the spirals show in their central portion an unstained area, where the substance of the spirochete is of smaller caliber, as if it were thinned out. In long forms, two, occasionally three, attenuated portions may be seen. This attenuation may be so marked at one point that it is difficult to say whether the continuity between the two portions is preserved or broken. Numerous spirochetes may also be found, lying end to end, with their tapering extremities in close approximation, strongly suggesting a recent separation.

These appearances, to our mind, force the conviction that reproduction in these spirochetes normally occurs by transverse fission, or possibly fragmentation. In spite of painstaking study, we have not succeeded in actually seeing this take place in living preparations. Because of their active motility, their low refractive index, and the difficulty in keeping the entire spiral in one optical plane, it is practically impossible to watch the same organism continuously for any length of time.*

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* On one occasion transverse division was observed, but, owing to the difficulty of being positive in the interpretation of such phenomena, the above conservative statement is advisable.

In stating that the spirochetes multiply by transverse division, we do not exclude the possibility that other modes of reproduction may take place. Of longitudinal division, or of sporulation, we have seen no evidence.

When the spirochetes are abundant, one frequently finds two or more organisms entwined, or, when very numerous, in tangled masses. They are, however, so often seen in certain definite relations to each other that one might be inclined to attribute to this a more than casual significance. We have been impressed with the frequency with which two spirochetes are seen entwined throughout their entire length, or lying side by side, accurately apposed, or even apparently fused through a portion of their length. The fused portions may terminate as a broad mass, ending in several short terminal filaments. We do not think that this appearance signifies a longitudinal division. These appearances remind one of the fusion which has been observed in the bacteria; or, again, it may be simply an expression of their tendency to adhere to each other under certain conditions. The spirochetes tend to adhere to the red blood cells, to the hemokonia or blood dust of Müller, to the blood plaques, and to various particles present in the blood. Finally, as a theoretical possibility, one may think of a conjugation analogous to that seen in the protozoa.

Extraordinary forms have been noted in the blood of a few rats, which require notice. In Rat 87 the spirochetes were found in great numbers. Many of the tangled masses were composed of individuals having the usual spiral appearance. Other masses were composed of more or less flattened or ribbon-like, and partly sinuous or even straight, individuals, these varied forms being more or less intimately united by faintly staining intercellular substance. Ribbon-like forms were quite common. In some of these thick forms an intervening clear space was made out, with the aid of compensation ocular No. 8. (See Plate 7, Fig. 4.) The ribbon-like forms may stain quite intensely, although usually they show a pale-blue color with the ordinary blood stains. Forms are not infrequently seen in which most of the protoplasm stains faintly, with several more deeply stained areas. The appearance occasionally suggests the presence of a macro- and micro-nucleus, but, on the whole, the more acceptable, and less strained, explanation is that, as in bacteria, irregularity in staining occurs. To us the ribbon-like forms are analogous to the so-called involution or degeneration forms of bacteria. The peculiar forms above described we have seen in rat blood, when the spirals are numerous, just before their final disappearance from the circulating blood. Under such conditions, it seems more reasonable to infer that these peculiar forms are the expression of degenerative or necrobiotic changes rather than developmental changes or evidences of longitudinal division.

STAINING REACTIONS.

Films have been stained with Wright's, Jenner's, Giemsa's, and Goldhorn's blood stains; with the ordinary aniline dyes, and with Gram's stain, the spirochetes decolorizing by the latter method; also with Loeffler's and with Proca's[11] flagella stain.

Wright's stain has been used for routine examination. With this stain the spirals are colored a blue of moderate intensity, or, if the differentiation is carried farther, a faint or even a pronounced red. The other polychrome stains have yielded, in our hands, no essentially different results. Hematoxylin stains them faintly, but uniformly.

With methylene blue, followed by Gram's iodine, the spiral is stained grayish black throughout its entire length, this being the color which the metachromatic granules of bacteria assume when stained by this method. The results obtained by the use of these stains—that is, a uniform, diffuse stain—speaks strongly against the assumption of the presence of a macro- and micro-nucleus. By means of aqueous methylene blue an intra-vital staining may be brought about in the hanging-drop, the spirals retaining their motility for 24 hours or longer.

MOTILITY.

In fresh hanging-drop preparations the most characteristic movement is a very active wavy or corkscrew one, broken by an asystolic interval of variable length.* This movement may apparently start from either end, and an alternation in the wave of contraction is always noted, except when a spiral becomes attached by one end to some object. A very definite lateral oscillation also takes place. This ranges from a slight trembling to a violent to-and-fro agitation of the entire spiral. At times a distinct and rapid forward-and-backward progression is seen, the total excursion sometimes covering half the microscopic field of an immersion lens.

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* For very simple optical reasons, it is impossible to decide whether this is an actual revolution about the long axis, or a rhythmic waving in one plane, comparable to the waving of a pennant in the wind.

In the hanging-drop kept for two days, either at room temperature or in the incubator, the spirochetes retain their various forms of movement. As their vitality becomes impaired, however, their movements grow less rapid, and often assume a tetanic character. When the corkscrew movements become slow, and occur at longer intervals, it can readily be made out that the motion in one direction originates at one extremity, the other extremity determining motion in the reverse direction.

DEGENERATIVE AND NECROBIOTIC CHANGES.

With this gradual loss of motility the spirochetes undergo a marked change in appearance. The threads become swollen and unevenly refractive, resembling the thick, ribbon-like forms found in stained blood smears. Later there are constantly found tenuous, beaded forms, which may be compared to some of the irregularly staining or moniliform individuals seen in the blood films. The presence of such forms only shortly precedes the final disappearance of the spirals from the citrated blood.

It is our impression, therefore, that these forms represent a stage in the dissolution of the spirochetes.

ATTEMPTS AT CULTIVATION.

Many attempts have been made to obtain cultures of the spirochetes, all of which have been unsuccessful. Basing our choice of media upon a possible relationship between these organisms and the trypanosomata, the 2 to 1 rabbit blood agar as recommended by Novy and McNeal[12] was tried. This medium, though tested a number of times, gave no results.

Various attempts were made to obtain cultures in fluid media. These media may be briefly mentioned: human blood with meat extract broth; human blood with human muscle broth; human blood with rabbit muscle broth; ascitic broth with and without blood. Inoculations on these media were negative. In some cases the blood, before being added to the broth, was mixed with sodium citrate solution, and in others the medium containing blood was heated for one-half hour at 55° C., to destroy the complement.

In some cases the blood was laked by citric acid, and subsequently neutralized by the addition of sodium carbonate.

Cultures were kept both at room temperature and in the incubator. In none of these fluid media was any evidence of multiplication observed, the spirals never being recovered.

Evidences of multiplication, however, were obtained in the condensation water of human and rat blood agar, the agar being made up with 2 per cent peptone and 1 per cent dextrose, and with human muscle infusion instead of the usual Liebig's meat extract. The condensation water, after 24 hours at room temperature, showed actively motile spirochetes in the hanging-drop, in greater numbers than in the original blood, as determined by the number seen in smears of the blood made at the time of inoculation. Transplants of a few loops from the condensation water to similar blood-agar tubes yielded practically negative results, although in many cases a few spirals were found on examination. The tubes kept at 37° C. uniformly gave negative results.

EVIDENCES OF MULTIPLICATION OF THE SPIROCHETES IN HUMAN AND RAT CITRATE BLOOD AT ROOM TEMPERATURE.

Positive evidences of the multiplication of the spirochetes in fluid media were observed in a number of experiments. In human and rat blood, to which has been added sodium citrate to prevent coagulation, there can be seen, within 24 hours after inoculation with a few drops of rat blood containing spirochetes, a very evident increase in the number of these organisms. By this we mean that, notwithstanding the dilution of several drops of infected blood with 60 to 150 times its volume, the spirochetes are more numerous in the smears from the culture fluid than in control smears taken at the time of inoculation.

Furthermore, after inoculating several drops of this first generation into a second blood tube, we have found the organism in approximately the same numbers in the second transplant.

The third generations, however, have failed to grow, although in the hanging-drop, non-motile, and in stained specimens, granular or moniliform organisms were seen. Although multiplication of these organisms in citrate blood was the rule, in a few cases increase was not observed. For instance, citrate blood, obtained from some patients, seemed to exert a deleterious influence upon the spirochetes, none being found in the blood after 24 hours. These variations in human blood may account for some of the negative observations. Multiplication likewise occurs in the undiluted citrate blood from infected rats, kept over night at room temperature, and in many cases tangled clumps develop.

The following brief description must suffice to justify the above statements upon the occurrence of multiplication:

Rat 51: December 4, inoculated with citrated blood of Rat 49, showing spirochetes. December 7, a few spirochetes found in stained smears. Rat was then etherized, and blood aspirated from heart. A few drops of blood introduced into a test tube containing 5 c.c. of human citrated (circa 3 per cent sodium citrate) blood. Tube kept at room temperature. In hanging-drop preparations, 19 hours after inoculation and in smears stained by Wright's stain, numerous single as well as tangled masses of spirochetes were seen. The drops for examination were taken from the surface of the somewhat turbid supernatant fluid. Transplants of a few drops from this tube into human citrate blood, on examination after 24 hours at room temperature showed actively motile spirals, not as numerous as in the first generation. Third generations were practically negative.

An agar flask containing 20 c.c. of Medium H* and 2 c.c. human citrate blood inoculated with a drop of infected rat blood, and kept for 19 hours at room temperature, showed numerous spirochetes in hanging-drop.

Rat 54: December 6, inoculated with blood of Rat 50, latter showing a few spirochetes. December 9, a few spirochetes found in smears made from blood of rat's tail; rat etherized, heart's blood aspirated, and mixed with citrate solution, and a tube containing 3 to 5 c.c. citrate human blood was inoculated with two drops of blood. After 24 hours at room temperature, the rat blood diluted with citrate solution showed numerous single and tangled masses of spirals in hanging-drop and in smears from fluid taken from surface of the settled blood tube. Transplants from this tube in sterile citrate rat blood were unsuccessful. In the citrate human blood tube no spirals were found. Plain human meat broth, and two tubes of human meat broth 5 c.c. + 1 c.c. human citrate blood, inoculated with several drops of blood of Rat 54, show no spirochetes on examination.

In this experiment there was an evident multiplication of the spirals in the original rat blood, no multiplication being observed in the dilutions.

Rat 55: As in Rat 54, there was an evident multiplication of the spirals in the original citrate rat blood after 24 hours at room temperature. No evidence of growth occurred in the citrate human blood tubes inoculated with a few drops of rat blood.

Rat 56: December 9, inoculated with blood of Rat 54, latter showing numerous

* Medium H:

Peptone	2	per cent
Sodium chloride	.5	" "
Dextrose	2	" "
Agar	2	" "

Slightly alkaline to phenolphthalein.

spirals. December 11, extremely numerous spirals were found. Citrate human blood (3–5 c.c.) tubes, and normal rat citrated blood (3–5 c.c.), were inoculated with several drops of the heart's blood of Rat 56, and the rest of the blood was citrated and kept at room temperature. After 18 hours the spirochetes had multiplied greatly in the original citrated blood (Rat 56). Many large clumps and numerous single spirals were seen in the hanging-drop and in stained smears made from the fluid. The normal rat and the normal human blood likewise showed extremely numerous and actively motile spirochetes. The spirals were found not only on the surface, but also in equal numbers at different levels of the fluid, proved by carefully drawing off the fluid in fine capillary pipettes. Transplants to normal rat blood showed in hanging-drop a few slightly motile, but beaded spirals. Transplants from this second generation remained negative.

This experiment indicates that in the first generation in normal rat blood rapid multiplication takes place, but that further growth is for some reason inhibited. It has been constantly observed that where laking of the citrated blood occurs, as in the second-generation tubes of this experiment, no multiplication takes place.

Rat 57: December 9, inoculated with blood of Rat 54. December 12, a few spirals found in one large smear. Rat etherized, blood citrated, and a few drops inoculated into human citrated blood. After 18 hours the spirochetes were found in considerably greater numbers in the culture tubes than in tubes containing the original citrated blood.

Transplants of a few drops to tubes of the same medium showed, after 24 hours, a few motile and non-motile spirals in hanging-drops and in smears. Transplants from these tubes were unsuccessful.

Rat 58: December 11, inoculated with blood of Rat 57. December 14, spirochetes found. Smears of the blood at time of etherization of rat showed two spirochetes after five minutes' search. Rat etherized, and blood inoculated into tubes, which, 24 hours later, gave the following results:

Tube 1,	normal citr.	rat blood, inoc. with	2 gtt.	Sp. not found.
“ 2,	“ “	“ “ “ “	2 “	Sp. moderate number.
“ 3,	“ “	“ “ “ “	2 “	Sp. numerous.
“ 4,	“ “	“ “ “ “	2 “	Sp. few.
“ 5,	“ “	“ “ “ “ (heated 30′ at 56° C.)	4 “	Sp. not found.
Tube 6,	normal citr.	rabbit blood, inoc. with	4 gtt.	Sp. not found.

Transplants were made as follows:

From tube 3: a few drops into	citrated rabbit blood—2 tubes “ rat “ 1 tube
From tube 2: a few drops into	citrated rabbit blood—2 tubes “ rat “ “

After 24 hours at room temperature, transplants from Tube 2 showed a few straight, typical, slightly motile and non-motile forms; while the original citrated blood (Rat 58), examined on the same day (48 hours), showed numerous active spirals. Transplants from Tube 2 negative.

The extreme scarcity of the spirochetes in the blood at time of killing of rat, when compared with numerous spirals found in the above blood tube at 48 hours, as well as in transplants, indicates active multiplication. In this experiment the rapid multiplication observed is confined to the tubes of the first generation. Something inimical to the growth and even preservation of the spirochetes makes its appearance.

Rat 59: December 11, inoculated with blood of Rat 57. December 14, spirochetes numerous. December 15, spirochetes numerous, rat etherized, several drops of blood inoculated into:

3 tubes of normal citrated rabbit blood.
3 " " " " rat " .

Cultures on the three rabbit blood tubes were negative, when examined 24 hours later. A moderate number of spirals were found in hanging-drops from the rat blood, and in one of these tubes (Tube 6; see Plate 7, Fig. 6), clumps of actively motile spirochetes were found after 24 hours. Several loops of this tube, transplanted into citrated rat blood, revealed six actively motile spirals in one hanging-drop, which, when the dilution is taken into account, may indicate only the number actually carried over.

Rat 64: December 17, inoculated with blood of Rat 62, latter showing numerous spirochetes. December 20, very numerous single spirochetes, and also small clumps found in stained smears.

Cultures made on three tubes of citrated normal rat blood, each tube being inoculated with one drop of spirochetal blood. Tubes examined after 24 hours at room temperature.

Tube 1, a few motile spirochetes found.
" 2, no spirochetes found (slight laking).
" 3, not examined " " .

Tubes after 48 hours at same temperature:

Tube 1, spirochetes in moderate numbers.
" 2, only one spirochete found in hanging-drop.
" 3, spirochetes in moderate numbers.

This experiment has been introduced for the purpose of demonstrating that the best cultural results (indicated by multiplication) are obtained with the blood of rats which show few spirochetes. (See above experiment, Rat 58.)

Considering the large numbers of spirochetes in the blood of Rat 64, no multiplication can be claimed in this experiment, even when the dilution of the blood is taken into account (1 drop in 3–5 c.c. of normal citrate rat blood).

Rat 66: December 20, inoculated with blood of Rat 64, showing spirochetes very numerous. December 22, spirochetes in moderate numbers. Cultures in normal citrate rat blood were negative, while spirochetal rat blood after standing 24 and 48 hours, showed numerous spirochetes, most of them motionless and in clumps.

Rat 83: January 8, inoculated with blood of Rat 81. January 10, a few spirochetes found in blood after search through many fields. Rat etherized, and two drops of blood inoculated into 5 to 6 c.c. of citrated normal rat's blood. Blood of Rat 83 citrated and distributed in two tubes. After 24 hours at room temperature, the three tubes showed extremely numerous and actively motile spirochetes, a dozen or more to the field of the hanging-drop, and after 48 hours showed clumps and spirochetes which had lost their motility. Transplants (two loops), made after 24 hours upon the same medium, show next day only a few spirochetes.

This experiment indicates not only that a multiplication of the spirals must have occurred in the original citrate rat blood, but that a much greater multiplication took place in the normal citrated rat blood inoculated with only two drops.

As an illustration that this multiplication by no means always occurs in the normal rat blood, the following experiment is given:

Rat 85: January 10, inoculated with blood of Rat 83. January 12, a few spirochetes found. Rat etherized. The citrated blood, and the normal citrated rat blood inoculated with a few drops, were examined 24 hours later. Only an occasional spiral was found in the citrated blood of Rat 85, the dilution being negative at this time and also later.

We believe that from these experiments we are justified in concluding that under favorable conditions, a multiplication of the spirochetes occurs outside of the body. This conclusion is based upon the experiments detailed above, the results of which may be summarized as follows:

If a few drops of spirochetal rat blood be inoculated into 3 to 5 c.c. of citrated blood (human or rat blood), the spirochetes are frequently found in greater numbers than in the original infected rat blood kept, under similar conditions, at room temperature; and in much greater numbers than in the circulating blood when the tubes are inoculated, since at this time their discovery often requires a prolonged and careful search.

That the spirochetes found in the fluid medium represent the individuals which have been transferred with the few drops of rat blood is opposed by the fact of their being more numerous than in the original citrated rat blood, and also by the fact that they are present in approximately equal numbers at different levels of the fluid.

Only three previous observers have noted an increase in the numbers of spirochetes under artificial conditions.

Multiplication in defibrinated blood was noted by Lachmann[13] in 1880. Three experiments illustrating this are cited by him. Whereas examination of the blood when withdrawn (at the onset of the symptoms) showed spirochetes to be either absent or present only in small numbers, they appeared to increase rapidly in the blood removed, so that on the following day 3 to 20 were found in each field.

Albrecht[14] also states that he has frequently observed multiplication, but gives no detailed observations. His account of the development of the spirochetes *in vitro*, from refractile particles in the blood, has not been substantiated and is doubtless incorrect.

Gerhardt[15] has also noted an increase in numbers in blood withdrawn during a paroxysm, as well as their appearance after 24 hours in blood taken just before the onset of the attack, at a time when no spirochetes were found.

All other observers, on the other hand, have denied the occurrence of this multiplication. (See Kolle u. Wasserman.[16])

GENERAL CONSIDERATIONS UPON THE IDENTITY AND THE BIOLOGY OF THE SPIROCHETES ABOVE DESCRIBED.

A discussion and review of the literature of this class of microorganisms, the spirochetes, would extend the limits of the article unnecessarily. Moreover, this topic has been excellently and fully presented by Wladimiroff,[17] and quite recently by Waldemar Löwenthal,[18] while references to the recent articles are found in the publication edited by Schaudinn.[19]

However, a few words may not be amiss in calling attention to the recognition of a certain number of new diseases in man and animals as being caused by species of this interesting class of microorganisms. Twenty-three years after the demonstration of the spirochete of relapsing fever by Obermeier[20] (1868; observation published 1873), Sakharoff[21] (1891) demonstrated the etiological connection of *Spirocheta anserina* with septicemia of geese. Marchoux and Salimbeni,[6] in 1903, published their observations upon the Brazilian disease of fowls, and have likewise established another spirochete, the *Sp. gallinarum*, as the pathogenic agent of this disease. Laveran,[22] in 1902, announced, for A. Theiler, the discovery of a spirochetal infection of cattle in South Africa, due to the *Sp. Theileri.* Martoglio and Carpano[23] have recorded a case of spirochetal infection in a sheep, the organism being essentially similar in morphology to the spirochete of Obermeier. By the observations of Ross and Milne,[24] Dutton and Todd,[8] of R. Koch,[25] and of other observers, the tick fever of man in Africa has been definitely decided to be due to a spirochete which greatly resembles in its morphology the *Sp. Obermeieri.*

C. Nicolle and C. Comte[26] describe briefly a spirochetal infection of bats in Tunis, which is of especial interest on account of the prevalence of relapsing fever in the Arabs of Tunis and the surrounding country. Monkeys and white mice are immune. In morphology it resembles, according to these observers, the spirals of relapsing fever. It has finely tapering extremities, stains easily, and divides by transverse division. All these spirochetal blood infections have one characteristic in common, namely, the constant presence in the circulating blood of the spirochetes during the course of the disease.

The morphology of these various spirochetes, according to different observers, is again strikingly similar. Thus, the description of *Sp. Theileri*, of *Sp. gallinarum* by Levaditi, of the spirochetes found in bats by Nicolle and Comte, of those found in sheep by Martoglio and Carpano, and the spirocheta of tick fever described by R. Koch, are in striking accord, and are not at variance with the morphological appearances presented by our spirochete. At the present time it is only by means of the animal reactions produced by this class of microorganisms that those producing the infections in man can be separated from those causing infections in animals. With these points in view, the *Sp. anserina* may be considered to be a species distinct from *Sp. gallinarum.*

While Dutton and Todd believe the relapsing fever of Europe and the tick fever of Africa to be identical, Koch has observed certain differences, such as the mildness of the clinical symptoms, and scarcity of the spirals in the circulating blood. As a result of his researches with the spirochete isolated by us, Professor Novy[9] believes that it is identical with the *Sp. Obermeieri.* Furthermore, he is inclined to believe that the relapsing fever of Europe and the tick fever of Africa are due to different spirochetes, on account of differences in the course of the spirochetal infection in white rats, as observed by Dutton and Todd, and by himself.

In contrast to the benign disease brought about in monkeys by the inoculation of our spirochete, the observations recorded by Dutton and Todd, and by R. Koch, show that the infections in their monkeys ran a more severe, or even fatal, course.

Infection in our monkeys has been constantly attended by the appearance of several paroxysms, while the disease caused in the same animals by the *Sp. Obermeieri* is characterized by the rarity with which relapses occur. Since inoculations in white rats with the latter organism have not been recorded, the infections of these animals described by Dutton and Todd, and by ourselves, cannot be claimed to be differential animal reactions.*

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* Several observers (Carter,[28] Karlinski[29]) state that mice are insusceptible: whereas Mamurowski[29] states that rats are naturally immune.

The question of the hour concerns itself with the classification of the spirochetes. Schaudinn believes that *Sp. pallida* and all other spirochetes are protozoa, and not bacteria. Vuillemin[27] has renamed the *Sp. pallida*, designating it *Spironema pallidum* on account of certain morphological appearances. This nomenclature was at first accepted by Schaudinn, but later, owing to the fact that the name *Spironema* is used in zoölogy for another form of life, he proposed the generic name *Treponema.*

According to Schaudinn, the coils of the *Sp. pallida* are permanent—a characteristic which serves, with the demonstration of a terminal cilium, to separate it from the true spirochetes, which have more or less blunted ends. The *Sp. pallida* is differentiated by Schaudinn from the spirilla by the presence of only one cilium instead of a terminal bush of cilia, by the flexibility of the spirals, and by the occurrence of longitudinal division. Although he considers this last point not positively established, nevertheless he believes the occasional demonstration of two terminal cilia to be an indication of approaching longitudinal division. He has been able to demonstrate in the living and in stained specimens a membrane or a periblast in all species of spirochetes, except the pallida. Lately, he is under the impression that he has detected a membrane around *Sp. pallida* in the fresh condition.

Following Schaudinn's argument, our spirochetes must be classed with *Sp. pallida.* The ends are invariably drawn out, and terminate in delicate filaments, quite as long and as delicate, if not more so, than the cilia he has described and depicted for the *Sp. pallida.* These thinned-out ends he terms cilia. But, as we have stated above, these terminal attenuations differ materially from the cilia of bacteria, since, unlike the latter, they appear to be direct prolongations of the body protoplasm, and, in accordance with this, have the same qualitative staining reactions. For these reasons, it seems to us more appropriate to call the tapering extremities of our spirochetes, as well as those of *Sp. pallida*, terminal filaments.*

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* Since writing the above section, there has appeared in the *Berl. klin. Wchnschr.*, 1906, No. 7, Feb. 12, a further communication from R. Koch, relating to the African recurrent fever. In this article Koch states that he has been unable to confirm the statements of Schaudinn upon the *Sp. Obermeieri.* He, like ourselves, has found no evidence of a blepharoplast, nucleus, or undulating membrane (Flimmersaum). Professor Zettnow, to whom Koch has referred for support concerning the morphological evidences on these points, has made the interesting observation that the terminal filament of the spirochetes unlike the cilia of bacteria, stain with methylene blue—an observation which confirms our own.

Furthermore, our spirochetes in rest and in motion retain their spiral coils.

We have already referred to our photographs which show the closest approximation to an indication of longitudinal division which we have observed in many hundreds of stained smears of rat, monkey, and human blood. It is not necessary to enter again into a discussion of our explanation of such appearances.

On account of the unsatisfactory evidence which the study of the morphology of this class of microorganisms has furnished, many investigators have made attempts to determine the nature of the spirochete by a study of its biological characteristics in those insects which are known to be the active agents in the transmission of the various spirochetal infections. The first observations seem to have been made by Tictin[31] upon the transference of relapsing fever to monkeys, by the injection of the crushed bodies of bed-bugs which had fed upon the blood of fever patients. These observations prove simply that the spirochetes remain alive a few days in the bed-bug.

Marchoux and Salimbeni have infected fowls by the bites of certain insects, the *Argas miniatus*, which have fed on spirochetal blood. Theiler believes that the African cattle fever is transferred by ticks under natural conditions. Dutton and Todd have lately shown that the tick is responsible for the transmission of the human tick fever. Ticks developed from eggs dropped by infected insects transmit the spirochetal infection to monkeys. These observations, and those made by Schaudinn upon the developmental stages of *Sp. Ziemanni*, coupled with the weight of his authority, have induced many to regard the spirochetes as protozoa. A careful examination of Dutton and Todd's paper shows that they have observed no developmental stages in the tick, which would justify them in their conclusion that the spirochetes undergo a cycle. They found the spirochetes unchanged in the stomach and in the malpighian tubes. On the other hand, Koch, it seems, has definitely shown that they undergo no developmental stages in the tick, but pass out of the stomach in a few days, and reach the ovary, where they multiply rapidly, and surround the eggs in tangled masses.

Borrel and Marchoux[32] have shown that the *Sp. gallinarum* remains alive and infective for a long time in the bodies of the *Argas* *miniatus*, when the insect is kept at a temperature of 35° C. The spirochete is found at first only in the stomach; later it pierces the stomach wall, and may be found in the general body cavity and in the ducts of the salivary glands. No evidence was obtained of a developmental cycle or transformation of the spirochetes within the bodies of this insect. These observations are in accord with the well-known viability of the *Sp. Obermeieri*, as shown by its remaining alive in capillary pipettes and in blood kept at room temperature for several days. Pasternazki,[33] fifteen years ago, showed that the spirochetes remain alive in the body of the leech for a considerable period of time, and, more recently, Karlinski[34] has found them alive in bed-bugs for thirty days after feeding on spirochetal blood.

The foregoing considerations as to the biological status of the spirochete, based upon our work and that of other observers, appear to warrant the following statement:

The absence of any definite indication of longitudinal division; the absence of such chromatic particles as justify belief in the existence of macro- and micro-nucleus; the unquestionable occurrence of transverse fission; the positive evidence of the production of active immunity, and, as shown by Professor Novy, the formation of antibodies in sufficient amount to lend passive immunity: these facts, the authors believe, indicate that the spirochetes must not be considered as protozoa.

On the contrary it would seem more rational, at the present time, to consider them to be either bacteria or in a class by themselves.

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EXPLANATION OF PLATE 7.

FIG. 1.—Usual type of spirochete. Human blood, Case 1. (Wright's stain; ×1,500.)

FIG. 2.—Rat 73. Two short forms, each with three spirals. The lower is greatly thickened. (Wright's stain; ×1,500.)

FIG. 3.—Rat 87. Numerous long forms, joined end to end, or partially approximated. Beginning agglutination (?). In the right upper quadrant two spirochetes are seen, closely opposed throughout the greater portion of their length. (Goldhorn's stain;* ×2,000.)

FIG. 4.—Rat 87. Very long form, consisting of three portions, separated by attenuated intervals. The upper and middle portions are considerably thickened, and ribbon-like. There appears to be a small, unstained cleft in the upper portion. The middle segment shows two deeply staining points, where the flattened spirochete is turned upon itself. (Goldhorn's stain; ×2,000.)

FIG. 5.—Rat 87. Two long spirochetes, end to end, apparently having recently divided. Each spirochete has a thinned-out central portion. here is a deeply staining granule on one of the organisms. (Goldhorn's stain; ×2,000.)

FIG. 6.—Rat 59. Tube 6. (Citrated normal rat blood; first generation, 48 hours.) Smeared with addition of ascitic fluid. Tangled clump showing tendency to radial arrangement. Moniliform degeneration of individual spirochetes. (Wright's stain; ×1,500.)

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* Goldhorn's recent modification of his polychrome methylene blue stain, described at the January meeting of the New York Pathological Society, 1906.

PLATE 7.

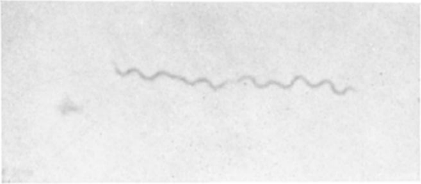

FIG. 1.

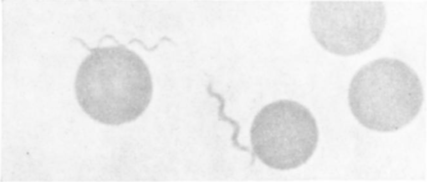

FIG. 2.

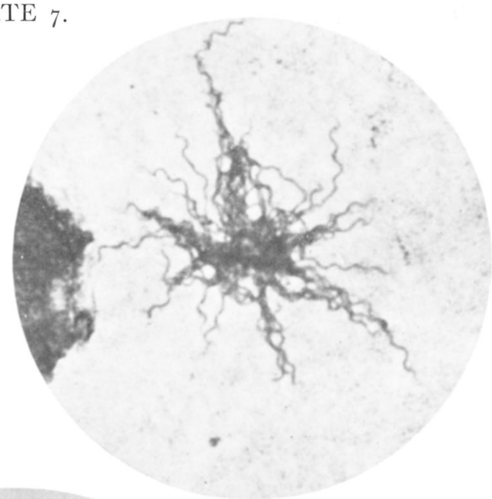

FIG. 6.

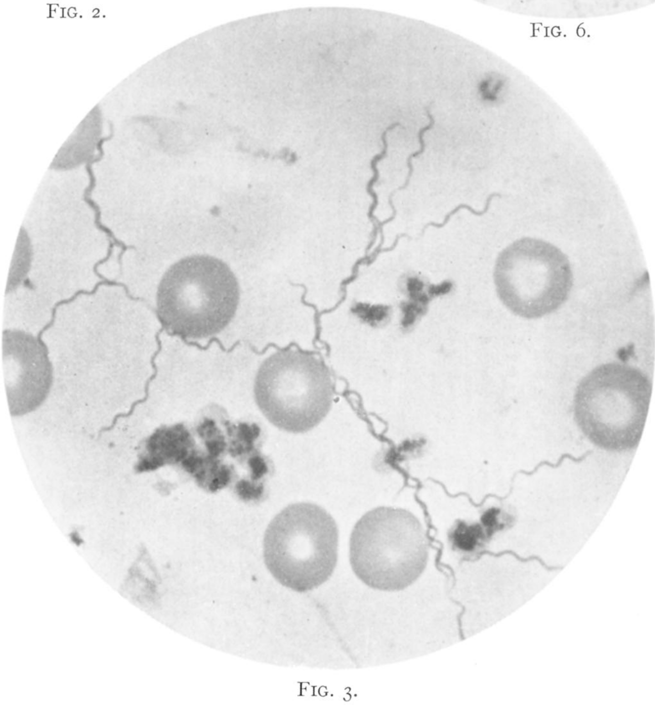

FIG. 3.

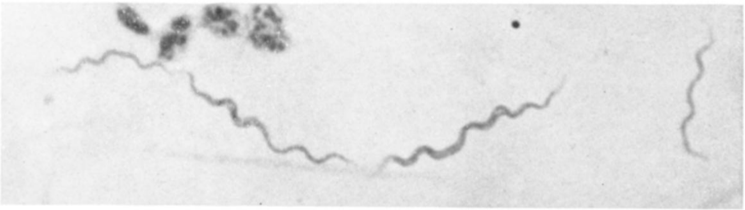

FIG. 4.

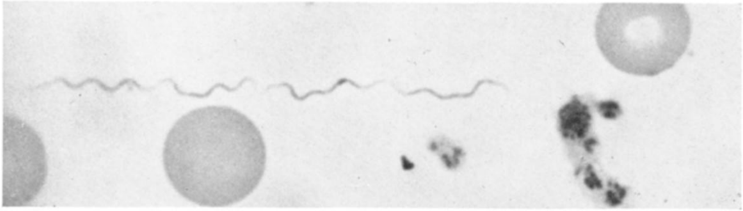

FIG. 5.